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"Growth Factor Involvement in Tension-Induced Skeletal Muscle Growth"

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I. SUMMARY OF PROJECT

Long-term manned space travel will require a better understanding of skeletal muscle atrophy which results from microgravity. Astronaut strength and dexterity must be maintained for normal mission operations and for emergency situations. Although exercise in space slows the rate of muscle loss, it does not prevent it. A biochemical understanding of how gravity/tension/exercise help to maintain muscle size by altering protein synthesis and/or degradation rate should ultimately allow pharmacological intervention to prevent muscle atrophy in microgravity. The overall objective of this research project is to examine some of the basic biochemical processes involved in tension-induced muscle growth. With an experimental in vitro system, we are examining the role of exogenous and endogenous muscle growth factors in mechanically stimulated muscle growth. Differentiated avian skeletal myofibers can be "exercised" in tissue culture using a newly developed dynamic mechanical cell stimulator device which simulates different muscle activity patterns. We have found patterns of mechanical activity which significantly affect muscle growth and metabolic characteristics. Both exogenous and endogenous growth factors are essential for tension-induced muscle growth. Exogenous growth factors found in serum, such as insulin, insulin-like growth factors, and steroids, are important regulators of muscle protein turnover rates and mechanically-induced muscle growth. Endogenous growth factors are synthesized and released into the culture medium when muscle cells are mechanically stimulated. At least one family of mechanically-induced endogenous factors, the prostaglandins, help to regulate the rates of protein turnover in muscle cells. Endogenously synthesized IGF-1 is another. We continue to study the interaction of muscle mechanical activity and these growth factors in the regulation of muscle protein turnover rates with our in vitro model system.

II. PROGRESS DURING FY 1992-93

Intermittent mechanical stimulation of cultured skeletal muscle cells for several days increases tension development and the amount of work performed by the muscle cells based on morphological and metabolic measurements, respectively. These forces stimulate myofiber hypertrophy, but only in the presence of serum growth factors. In basal medium without serum, mechanical stimulation slows the rate of myofiber atrophy but does not induce hypertrophy. The serum growth factors necessary for stretch-induced growth are not known. Protein synthesis rates are stimulated by mechanical activity in both media, but to a greater extent in serum medium than in unsupplemented media. Long term protein degradation rates respond differently in the two media. In serum-containing medium, mechanical stimulation reduces the rate of long lived protein degradation; in basal medium, protein degradation rates are accelerated by mechanical stimulation. These differences in protein turnover in basal versus serum supplemented media can thus explain why hypertrophy occurs in one but not the other situation.

INSULIN AND IGF-1

In order to analyze which growth factors are essential for stretch-induced muscle growth in vitro, we developed a defined, serum-free medium in which the differentiated, cultured muscle fibers can be maintained for extended periods. The defined medium (muscle maintenance medium, mm medium) maintains the nitrogen balance of the myofibers for 5 to 7 days, based on myofiber diameter measurements and myosin heavy chain content. Insulin and insulin-like growth factor-1, but not insulin-like growth factor-2, induce rapid and pronounced myofiber hypertrophy when added to this medium. In 5 to 7 days, muscle fiber diameters increase by 71% to 98%.

Mechanical stimulation of the muscle fibers in mm medium increases the sensitivity of the cells to insulin and insulin-like growth factor-1 (IGF-1), based on a leftward shift of the insulin dose/response curve for protein synthesis rates. Thus, one mechanism by which mechanical activity stimulates myofiber growth is by increasing the sensitivity of the cells to these growth factors. This mechanism is compatible with the known beneficial effects of exercise in patients with diabetes. The intracellular signalling mechanisms by which muscle stretch increases their sensitivity to insulin and IGF-1 are unknown.

One possibility is that stretch increases the production of endogenous IGF-1. We developed an RIA for IGF-1 and measured synthesis by the cultured muscle cells. We found that the differentiated skeletal muscle cells release large amounts of IGF-

1 into the culture medium (1-3 nM) under static culture conditions. When mechanically stimulated, the rate of IGF-1 release into the medium is significantly increased but only during the first hours after initiating mechanical stimulation (Figure 1). Longer periods of stimulation lead to a significant decrease in IGF-1 release from the cells. We have tested several different mechanical activity patterns with similar results. This type of IGF-1 stretch response is compatible with a model whereby stretch increases the secretion but not the de novo synthesis of IGF-1. Understanding the mechanism by which mechanical forces modify the IGF-1 secretion rate of muscle fibers may lead to new therapeutic treatments for the muscle atrophy resulting from decreased tension development occurring in the microgravity of space.

Skeletal muscle cells also synthesize and secrete IGF-1 binding proteins which can influence the growth-stimulating properties of IGF-1. We developed a ligand binding assay for IGF-1 binding proteins and found that the skeletal muscle cultures produce three major species of 31, 36, and 43 kDal molecular weight. Preliminary experiments indicate that stretch may increase the release of the 36 kDal binding protein, which might modulate IGF-1 growth stimulating activity. These experiments are currently being extended.

STEROIDS

Steroid hormones have a profound effect on muscle protein turnover rates with the stress-related glucocorticoids inducing rapid skeletal muscle atrophy while androgenic steroids induce skeletal muscle growth. Exercise in humans and animals reduces the catabolic effects of glucocorticoids and enhances the anabolic effects of androgenic steroids on skeletal muscle. In our continuing work on the involvement of exogenous growth factors in stretch-induced skeletal muscle growth, we have performed experiments to determine whether mechanical stimulation of cultured muscle cells alters their response to anabolic steroids or glucocorticoids. In static cultures, testosterone has no effect on muscle cell growth, but 5α-dihydrotestosterone and the synthetic steroid stanozolol increase cell growth by 18% to 30% after a three day exposure. We have been unable to find any alteration in the muscle cell's growth response to these steroids with stretch.

The glucocorticoid dexamethasone induces atrophy of the differentiated cultured myofibers after 3 to 5 days of incubation. Mechanical stimulation of the muscle cells for three days in the presence of dexamethasone significantly attenuates this atrophic response, based on a 79% reduction in the dexamethasone-induced fall in protein/DNA ratios and myosin content. Thus, mechanical stimulation modifies the response of the muscle cells not only to

insulin and IGF-1 but also to glucocorticoid hormones. We have recently extended these observations to determine whether stretch attenuates dexamethasone induced muscle atrophy by a prostaglandin-dependent mechanism. Dexamethasone inhibits the production of the anabolic prostaglandin $F_{2\alpha}$ in static muscle cultures and mechanical stimulation attenuated this decrease. The stretch-induced increase in $PGF_{2\alpha}$ resulted partially from stretch activation of the enzyme responsible for synthesizing prostaglandins, cyclooxygenase. Thus, mechanically-induced protection of muscle fibers from the catabolic effects of stress-related hormones such as the glucocorticoids acts at the level of cyclooxygenase and could be an important mechanism by which exercise protects skeletal muscle from stress-related atrophy in space. Understanding the molecular mechanism of stretch-regulated cyclooxygenase activity could lead to the development of beneficial pharmacological agents in the treatment of muscle wasting.

COMPUTERIZATION OF THE MECHANICAL CELL STIMULATOR

We have made progress toward developing a new IBM based hardware and software system to provide greater flexibility in operating and monitoring the mechanical cell stimulator. This system is presently in beta testing. These alterations are aimed at providing both a better ground-based model system for studying skeletal muscle growth regulation and as a model system for space station studies.

III. PUBLICATIONS DURING FY 1992-93

FULL PAPERS

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2. Vandenburgh,H.H., Hatfaludy,S., P. Karlisch, and J. Shansky (1992) Mechanically induced alterations in cultured skeletal muscle growth. J. Biomech. 24, 91-101.

3. Chromiak, J.A. and Vandenburgh, H.H. (1992) Glucocorticoid-induced skeletal muscle atrophy in vitro is attenuated by mechanical stimulation. Am. J. Physiol. 262, C1471-C1477.

4. Vandenburgh, H.H., Shansky,J., Karlisch,P., and Solerssi,R. (1993) Mechanical stimulation of skeletal muscle generates lipid-related second messengers by phospholipase activation. J. Cell. Physiol. 155, 63-71.

5. Shansky,,J. Karlisch, P., and Vandenburgh, H.H. (1992) Skeletal muscle mechanical cell stimulator. In Protocols in Cell and Tissue Culture (J.B. Griffiths, A.Doyle, and G. Newell, eds.), John Wiley and Sons Limited, Chichester, In Press.

6. Vandenburgh, H.H., Shansky, J., and Solerssi, R. (1993) Mechanical stimulation of skeletal muscle in vitro increases prostaglandin $F_{2\alpha}$ snthesis and cyclooxygenase activity by a pertussis toxin sensitive mechanism. Submitted.

7. Chromiak, J.A. and Vandenburgh, H.H. (1993) Mechanical stimulation of skeletal muscle mitigates glucocorticoid-induced decreases in prostaglandin synthesis. Submitted.

ABSTRACTS

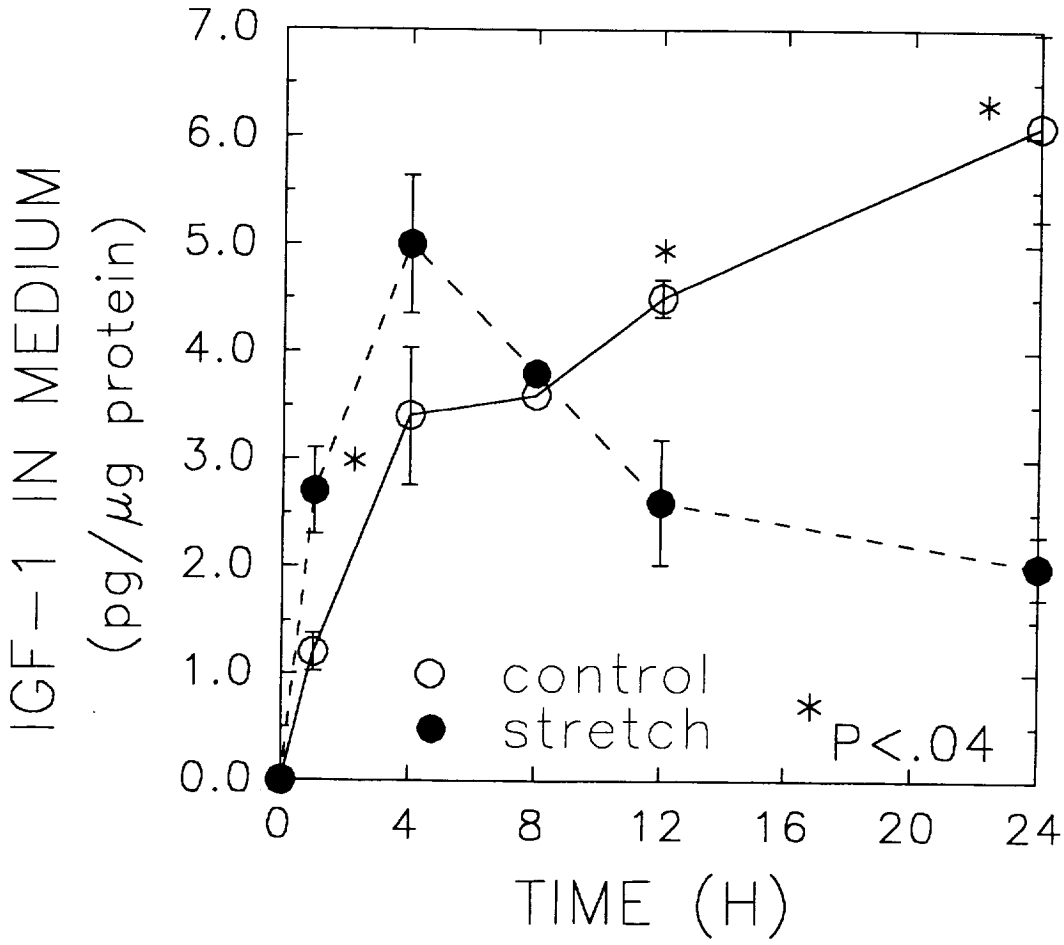
1. Chromiak, J.A., Vandenburgh, H.H., Shansky, J., Solerssi, R. (1992) Repetitive mechanical stimulation of tissue cultured skeletal muscle mitigates glucocorticoid-induced decreases in $PGF_{2\alpha}$ synthesis and prostaglandin H synthase activity. Physiologist 35, 213.

2. Vandenburgh, H.H., Solerssi, R., and Fenwick-Smith, D. (1992) Insulin-like growth factor-1 as an anabolic regulator of tension-induced skeletal muscle growth ASGSB Bulletin 6, 93a.

3. Vandenburgh, H.H., Shansky, J., and Solerssi, R. (1992) Mechanical stimulation of skeletal muscle in vitro increases prostaglandin $F_{2\alpha}$ synthesis and cyclooxygenase activity by a pertussis toxin-sensitive mechanism. Mol. Biol. Cell 3, 244a.

4. Vandenburgh, H.H., Solerssi, R., Henderson, S., and Adams, J. (1993) Repetitive stretch of neonatal rat cardiomyocytes in vitro stimulates their rate of binucleation. Conf. on Mol. Biol. of the Normal, Hypertrophied, and Failing Heart, 38a.

FIGURE 1. STRETCH AND IGF-1 RELEASE



IGF1STR.SPG